BORANG PENGESAHAN STATUS THESIS

JUDUL: PRODUCTION OF BIODIESEL FROM PALM OIL USING ENCAPSULATED
LIPASE DERIVED FROM K-CARRAGEENAN.

IJAZAH: DOKTOR FALSAFAH

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PRODUCTION OF BIODIESEL FROM PALM OIL USING ENCAPSULATED LIPASE DERIVED FROM K-CARRAGEENAN

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SCHOOL OF ENGINEERING AND INFORMATION TECHNOLOGY
UNIVERSITI MALAYSIA SABAH
2010
DECLARATION

I hereby declare that the materials in this thesis are original except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

24 June 2010

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DEGREE : DOCTOR OF PHILOSOPHY (CHEMICAL ENGINEERING)
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Acknowledgement

It is my pleasure to express my sincere thanks to all those respectful personalities who played supportive role for me during this research.

First of all I would like to express my deep sense of gratitude and respect to my supervisor Professor Dr. Pogaku Ravindra for his valuable full hearted guidance, confidence and constant encouragement. Prof. has always time for discussions, full of encourage and patience. Thank you Prof. for your generous scientific and practical knowledge as well as for letting me become a PhD student in an interesting and promising field.

I would like to thank my co-supervisor Dr. Eng Seng Chan for taking on the duty, kindness and help. Your encouragement and support were very reviving.

More special thanks are dedicated to the members of the encapsulation research group for many help during my research in the lab.

I am grateful to all my friends in Universiti Malaysia Sabah for their help and togetherness. I also would like to thank all people in School of Engineering and Information Technology for a nice working atmosphere.

I am highly indebted to Prof's family members for their care, help, and hospitality.

Admiration is dedicated to my parents, brothers, and family members for endless support and hopes. Your great love and care are unforgettable.

Finally, I acknowledge with thanks to the financial support provided by the MOSTI and FRGS.
ABSTRACT

PRODUCTION OF BIODIESEL FROM PALM OIL USING ENCAPSULATED LIPASE DERIVED FROM K-CARRAGEENAN

The objective of this research was to produce biodiesel using encapsulated lipase in an immobilized bioreactor. K-carrageenan was used as a matrix for encapsulating lipase PS from Burkholderia cepacia and the coextrusion technique was adopted to immobilize lipase. This study has been undertaken due to the low cost, non toxic, environmentally benign characteristics of K-carrageenan and the novelty of coextrusion technique. The physicochemical studies were conducted by using Microscope, Spectrophotometer SEM, FTIR etc. The results showed the diameter of the encapsulated lipase was in the range of 1.3-1.8 mm with an average membrane thickness of 200 µm. The encapsulation efficiency was found to be 42.6 percent. The optimum stability was observed at pH 7 and at temperature 40°C. The Immobilized lipase retained 72.3 percent of its original activity after using it for 5 cycles of reuse in hydrolysis of p-NPP.

Immobile encapsulated lipase was taken in stirred tank batch immobilized bioreactor (STIBR) and packed bed bioreactor (PBBR). The studies were carried out in a batch mode of operation and various process parameters were optimized for biodiesel production. HPLC was used for analyzing the biodiesel. The optimum conditions for processing palm oil in a stirred tank immobilized bioreactor (STIBR) were 30°C, 72 h reaction time and 23.7 x g relative centrifugal force. Similarly, the optimal conditions for processing palm oil in a PBBR were 1.5 ml/min and 264 h reaction time. STIBR showed conversion of up to 100 percent and the PBR has shown conversion up to 82 percent. Since the STIBR has higher conversion rate, the kinetic parameters \( K_m \) and \( V_{max} \) were evaluated and found to be 600 mol.m\(^{-3}\) and 0.84 mol.m\(^{-3}\).min\(^{-1}\) respectively. The kinetic parameter values were substituted into Michaelis–Menten empirical equation and the batch time was found to be the same as experimental value of 72 h. The encapsulated lipase retained 82 percent relative conversion after 5 cycles of reuse. The economic assessment of biodiesel production using immobilized enzyme catalyst process was challenging compared to the current alkali process. The Life Cycle Analysis (LCA) studies showed that biodiesel production using immobilized enzyme catalyst has lesser impact on the environment compared to the alkali catalyst and soluble enzyme catalyst. Based on the experimentation and the results, it is concluded that biodiesel production using encapsulated lipase in an immobilized bioreactor open new vistas for the scale up studies of this technology in near future.
Objektif kajian ini adalah untuk menghasilkan biodiesel menggunakan kaedah pengkapsulan lipase dalam bioreaktor amobil. κ-carrageenan digunakan sebagai median pengkapsulan Lipase PS daripada Burkholderia cepacia dan aplikasi kaedah pembentukan (coextrusion) untuk menghasilkan lipase amobil. Kaedah ini dipilih kerana ianya memerlukan kos yang rendah berbanding kaedah lain, tidak bertoksik penggunaan karrageenan yang bercirikan mersa alam dan novel dalam teknik pembentukannya. Kajian kimia-fizikal dilakukan menggunakan Mikroskop Spektometer (SEM), dan FTIR. Keputusannya menunjukkan bahawa diameter lipase yang pengkapsulan berada dalam linkungan 1.3mm hingga 1.8mm dengan purata ketebalan membar setebal 200 μm. Kebersihan proses kapsulasi adalah sebanyak 42.6 peratus. Kestabilan optimum telah diperhatikan pada nilai pH 7, pada suhu 40°C. Kecekapan aktivitinya sebanyak 72.3 peratus berbanding kecekapan asal diperhatikan dalam lipase amobil walaupun selepas 5 kitaran guna semula menggunakan hidrolosis p-NPP.

Kapsul lipase amobil dikeluarkan daripada siri bioreaktor yang dikacau (STIBR) dan batas bioreaktor berbungkus (PBBR). Kajian dijalankan dalam beberapa siri operasi dan melibatkan pengoptimum proses bagi pelbagai parameter untuk menghasilkan biodiesel. HPLC digunakan untuk menganalisis biodiesel. Persekitaran optimum bagi pemprosesan minyak kelapa sawit dalam siri bioreaktor yang dikacau (STIBR) adalah pada 30°C selama 72 jam pada 23.7 x g kelajuan relatif penghempanan. Keadaan optimum bagi pemprosesan minyak kelapa sawit dalam PBBR adalah 1.5 ml/min dan 264 jam untuk masa reaksi. STIBR menunjukkan 100 peratus penukaran berbanding 82 peratus menggunakan PBR. Memandangkan STIBR menunjukkan kadar pertukaran yang lebih tinggi, parameter kinetik Kₐₘ dan Vₘₐₓ dievaluasi dimana 600 mol.m⁻³ dan 0.84 mol.m⁻³.min⁻¹ diperolehi. Parameter kinetik yang dikira digantikan dalam persamaan impirik Michaelis-Menten, di dapati siri masa adalah sama dengan nilai rekod semasa ekperimen dijalankan iaitu 72 jam. Lipase yang pengkapsulan masih mengekalkan 82 peratus daripada penukaran relatif asalnya walaupun selepas 5 kitaran guna semula. Penilaian ekonomi dalam menghasilan biodiesel menggunakan proses katalisis enzim amobil merupakan kaedah yang lebih mencabar berbanding kaedah proses alkali seperti yang digunakan kini. Kajian analisis kitaran hidup (LCA) telah menunjukkan bahawa penghasilan biodiesel menggunakan katalisis enzim membawa impak yang lebih rendah kepada alam sekitar berbanding kaedah katalisis alkali dan katalisis enzim berlarut. Berdasarkan kajian ini dan keputusan yang diperolehi, dapat disumpulkan bahawa penghasilan biodiesel menggunakan kapsul lipase dengan kaedah pembentukan dalam bioreaktor amobil merupakan pendekatan baru untuk diterokai dengan lebih mendalam dalam bidang ini.
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### Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$</td>
<td>Boltzmann constant, kg m$^2$/s$^2$</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature, °C</td>
</tr>
<tr>
<td>$t$</td>
<td>Time, h</td>
</tr>
<tr>
<td>$E$</td>
<td>Activation energy, J/mol</td>
</tr>
<tr>
<td>$R$</td>
<td>Gas constant, 8.314 J/mol °K</td>
</tr>
<tr>
<td>$V$</td>
<td>Reaction rate constant or velocity constant</td>
</tr>
<tr>
<td>$D_e$</td>
<td>Diffusivity, m$^2$/s</td>
</tr>
<tr>
<td>$r$</td>
<td>Particle radius, m</td>
</tr>
<tr>
<td>$r_s$</td>
<td>Capsule outer core radius, m</td>
</tr>
<tr>
<td>$r_i$</td>
<td>Capsule inner core radius, m</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum reaction rate, mol/m$^3$ sec (transeserification)</td>
</tr>
<tr>
<td>$V'_{\text{max}}$</td>
<td>Maximum reaction rate, U/mg-protein (hydrolysis)</td>
</tr>
<tr>
<td>$V''_m$</td>
<td>Apparent Maximum reaction rate, mol/m$^3$ (catalyst).sec</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michalis Menten constant, mol/m$^3$ (transeserification)</td>
</tr>
<tr>
<td>$K'_m$</td>
<td>Michalis Menten constant, mmol (hydrolysis)</td>
</tr>
<tr>
<td>$C_S$</td>
<td>Concentration of the substrate at time $t$, mol/m$^3$</td>
</tr>
<tr>
<td>$C_{S0}$</td>
<td>Concentration of the substrate at time $t=0$, mol/m$^3$</td>
</tr>
<tr>
<td>$V_o$</td>
<td>Frequency factor or pre-exponential factor</td>
</tr>
<tr>
<td>$+r_p$</td>
<td>Rate of product formation, mol/m$^3$ (methyl esters)</td>
</tr>
<tr>
<td>$-r_s$</td>
<td>Rate of disappearance of substrate, mol/m$^3$ (triglycerides)</td>
</tr>
<tr>
<td>$A_{410\text{nm}}$</td>
<td>Absorbance, 410 nm</td>
</tr>
<tr>
<td>$\Delta\varepsilon$</td>
<td>Molar extinction of nitrophenol</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Effectiveness factor</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>Thiele modulus</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Michalis constant dimensionless number</td>
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CHAPTER 1

INTRODUCTION

The availability and environmental impact of energy resources will play a critical role in the progress of the world’s societies and the physical future of our planet. Worldwide energy consumption is increasing exponentially (Figure 1.1) and at present usage rates, these sources will soon be exhausted (Srivastava and Prasad, 2000), contributed to soaring fossil fuel prices. The majority of human energy needs are currently met using petrochemical sources, coal and natural gases. As the demand for energy has grown, so have the adverse environmental effects of its production.

Figure 1.1: World marketed energy consumption.
Source: IEA outlook (2007)

Emissions of CO₂ (Figure 1.2), SO₂ and NOₓ from fossil fuel combustion are the primary causes of adverse environmental effects (Ture et al., 1997). The accumulation of carbon dioxide and other greenhouse gases in the atmosphere is
thought to be responsible for climate change, which is predicted to have disastrous global consequences for life on this planet (Sheehan et al., 1998). Renewable energy may offer an excellent alternative to the fossil fuels, representing a cornerstone to steer our energy system in the direction of sustainability and supply security. Hence, Renewable energy sources have become a high priority in the energy policy strategies at national level as well as at a global scale.

**Figure 1.2: World energy-related carbon dioxide emissions by fuel type**
Source: IEA outlook (2007)

### 1.1 Renewable Energy

Renewable energy sources are indigenous, and can therefore contribute to reduce dependency on oil imports, increasing security of supply and environmental benefit. Due to these reasons, the investment towards renewable energy is drastically increasing around the world (Figure 1.3). Renewable energy can be classified into various types (Figure 1.4), among which biofuels could be considered as a major energy source promoted and produced in most of the countries around the world.
Figure 1.3: Global investment in renewable energy.
Source: REN21 (2009)

Figure 1.4: Classification of renewable energy.
1.2 Biofuels

Biofuels provide the prospect of new economic opportunities for people in rural areas, concerning job creation, greater efficiency in the general business, and protection of the environment (Demirbas, 2008)(Figure 1.5). Biofuels – liquid or gaseous fuels derived predominantly from biomass may be able to provide an alternative source of energy that could be both sustainable and without serious environmental impact. Biofuels are produced from plant oils, algal oil, animal fats, sugar beets, cereals, organic waste and the processing of biomass. The extent to which biofuels can ultimately replace fossil fuels depends on the efficiency with which they can be produced (Malcaaa and Freire, 2006). Biofuel research and deployment has intensified in all countries as an alternative to fossil fuel.

Figure 1.5: Benefits of biofuels.
Source: Demirbas, 2008
Global biofuel production has tripled from 4.8 billion gallons in 2000 to about 16.0 billion in 2007 with the US and Brazil contributing 75% of world production. Biofuels include bioethanol, biodiesel, biogas, bio-synthetic gas (bio-syngas), bio-oil, bio-char, Fischer-Tropsch liquids, and biohydrogen. Among these biodiesel is predominant and the biodiesel production is booming worldwide, with Europe accounting for the by far largest share of the global biodiesel production (Bacovsky et al., 2007).

1.3 History of Biodiesel
A relatively common literature statement on the early use of vegetable oils as diesel fuels is that of Rudolf Diesel, the inventor of the engine that bears his name tested “his” engine on peanut oil at the 1900 World’s Fair in Paris (Knothe et al., 2005). He quotes,

“In any case, they make it certain that motor-power can still be produced from the heat of the sun, which is always available for agricultural purposes, even when all our natural stores of solid and liquid fuels are exhausted.”

—Diesel, 1900

Initially, Rudolf Diesel was interested in running his engine on either coal or vegetable-based fuels. But, Petroleum-based fuels became the main source due to lower cost over the past century (Caye et al., 2008).

Vegetable oils were also used as emergency fuel during World War II. For example, Brazil prohibited the export of cottonseed oil in order to substitute it for imported diesel fuel. Reduced imports of liquid fuel were also reported in Argentina, necessitating the commercial exploitation of vegetable oils. China produced diesel fuel, lubricating oils from tung and other vegetable oils. However, the exigencies of the war caused hasty installation of cracking plants based on fragmentary data. Researchers in India, prompted by the events of World War II, extended their investigations on ten vegetable oils for development as a domestic fuel. Later, work on vegetable oils as diesel fuel ceased in India when petroleum-based diesel
fuel became available plentifully at low cost. The Japanese battle ship Yamato used edible refined soybean oil as bunker fuel. These events narrate the usage of vegetable oil as fuel for energy purpose in those days. However, the limitation of using vegetable oil as a fuel was its higher viscosity due to the presence of glycerol. The glycerol can be separated by a process known as transesterification (Knothe et al., 2005).

The principles of biodiesel production from vegetable oil by transesterification process have been known for a century. In 1937, The Belgian patent 422, 87 to G. Chavanne (of the University of Brussels), constitutes the first report on what is today known as biodiesel. It describes the use of ethyl esters of palm oil as diesel fuel. Even though biodiesel was patented in 1937, it was not commercially attractive due to the availability of petroleum based diesel fuel at low cost. Only after the oil crisis in 1973, research on methyl ester production technology and its application in diesel engines initiated worldwide. In Austria and France, stake holders from agriculture and industry were inspired and interest of investors arose. Pilot projects were conducted in both countries by the end of the 1980’s. One of the most important results was the publication of the world’s first standard for rape oil methyl ester, which laid the basis for the approval of fatty acid methyl esters as a transport fuel and a number of international standards. Currently there are many industries producing biodiesel around the world from various oil sources (Knothe et al., 2005).

### 1.4 Global Biodiesel Production

Biodiesel is produced from the transesterification of vegetable oils. Current global production of biodiesel is approximately 12 million tonnes (Figure 1.6) and the biodiesel production capacities have increased in 2008 compared to 2007 (Figure 1.7). These figures are likely to increase further, due to the implementation of 20% blend of biofuels in conventional diesel fuel in many countries. In addition, the recent biofuel policy 20:20:20 (20 percent cut in greenhouse gas emissions for all energy compared with 1990 levels, a 20 percent increase in the use of renewable energy and a 20 percent cut in energy consumption through improved energy...
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